



Plant and sediment properties in seagrass meadows from two Mediterranean CO₂ vents: Implications for carbon storage capacity of acidified oceans

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ABSTRACT

Assessing the status of important carbon sinks such as seagrass meadows is of primary importance when dealing with potential climate change mitigation strategies. This study examined plant and sediment properties in seagrass meadows (*Cymodocea nodosa* (Ucria) Asch.) from two high pCO₂–low pH Mediterranean vent systems, located at Milos (Greece) and Vulcano (Italy) Islands, providing insights on carbon storage potential in future acidified oceans. Contrary to what has been suggested, carbon content (both inorganic and organic) and its surficial accumulation decreased at high pCO₂–low pH in comparison with controls. The decrease in inorganic carbon may result from the higher solubility of carbonates due to the more acidic conditions. At Vulcano, the seagrass properties (e.g., leaf area, biomass) appeared negatively affected by environmental conditions at high pCO₂–low pH conditions and this may have had a detrimental effect on the organic carbon content and accumulation. At Milos, organic carbon decreased at high pCO₂–low pH conditions, despite the increase in seagrass aboveground biomass, leaf length and area, probably as a consequence of site-specific features, which need further investigation and may include both biotic and abiotic factors (e.g., oligotrophic conditions, decreased sedimentation rate and input of allochthonous material). Results suggest that, in contrast to previous predictions based exclusively on the expected positive response of seagrasses to ocean acidification, carbon storage capacity of the seagrass *C. nodosa* may not increase at high pCO₂–low pH conditions. This study emphasizes the need to investigate further the potential alteration in the climate mitigation service delivered by seagrass meadows in acidified oceans.

1. Introduction

By tempering the atmospheric CO₂ rise, oceans concurrently undergo a progressive decline in seawater pH, which is termed Ocean Acidification (OA) (Doney et al., 2009), one of the most well-known climate change effects, along with global warming. Combating climate change is a high priority nowadays, especially through a range of possible mitigation strategies to remove carbon, including the protection and restoration of globally significant carbon sinks (Duarte et al., 2013). Although accounting for a very small part of the ocean, marine vegetated ecosystems such as seagrasses, mangroves and salt marshes have high potential value for carbon sequestration and mitigation of the detrimental effects of climate change (Duarte et al., 2013; Marbà et al., 2015). More specifically, the total carbon that can be buried through

biosequestration within coastal oceans compares favorably with terrestrial ecosystems (Macreadie et al., 2014). Efforts to understand the mechanisms underpinning this important capacity have grown in recent years and belong to the new field of research known as “blue carbon”, which refers to the carbon that can be stored in coastal vegetated habitats (Duarte et al., 2005; Campbell et al., 2014; Fourqurean et al., 2012; Howard et al., 2014; Gullström et al., 2018).

Recent studies have drawn attention to the capacity of seagrass beds to act as efficient and especially fast CO₂ sinks (e.g., McLeod et al., 2011; Nellemann et al., 2009) and have investigated the properties of both sediment and plants that drive carbon storage in these ecosystems (e.g., Dahl et al., 2016). Seagrasses form a dense stratum, composed of a mixture of sediment, roots and rhizomes, which for a few species can be several meters in depth (Nellemann et al., 2009, and references

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therein). Given that the storage capacity of seagrasses is very high, between 4.2 and 8.4 Pg C (Fourqurean et al., 2012), and that the carbon within the sediments can be stored over millennia (see Duarte et al., 2005, and therein references), seagrasses play a major role as C sinks.

In the scenario of OA, it has been suggested that seagrass biomass will most likely increase, along with seagrass cover, enhancing organic matter burial and the potential for carbon storage and sequestration (Garrard and Beaumont, 2014). Accordingly, significant increases in seagrass shoot density and/or biomass have been reported both at temperate (Hall-Spencer et al., 2008) and tropical CO₂ vents (Fabricius et al., 2011; Russell et al., 2013), which are considered analogues of future high pCO₂-low pH environments, suggesting beneficial effects of OA on the capacity of seagrasses to act as carbon sinks under high pCO₂-low pH conditions (Takahashi et al., 2016). Seagrasses are indeed considered as potential “winners” in future oceans: while they are carbon limited at the current pCO₂, an increase in inorganic carbon, coupled with the low pH-driven reduction in encrusting calcifying epiphytes, is expected to boost photosynthetic rates and increase energy reserves (i.e., non-structural carbohydrates) (Campbell and Fourqurean, 2013; Zimmerman et al., 1997). However, research findings from vents are not always consistent with this conceptual outline. The effect of high pCO₂-low pH from vents on seagrass biomass, for example, is controversial, with Apostolaki et al. (2014) showing decrease in biomass of *Cymodocea nodosa* in the Vulcano vent (Mediterranean), while Russell et al. (2013) reported increased biomass of the con-generic *C. serrulata* in vents of Papua New Guinea. In this sense, a diverse response of seagrass to OA in terms of their capacity to capture carbon could be expected.

In this study we examined plant and sediment properties which may influence carbon storage capacity in seagrass meadows from two Mediterranean CO₂ vents to test whether there is a consistent response to high pCO₂-low pH and to infer potential implications for carbon sequestration and storage capacity in future acidified oceans. We expect that if seagrasses benefit from high pCO₂-low pH conditions, their increase in biomass and density should promote the accumulation of sedimentary carbon and hence carbon storage capacity.

2. Materials and methods

2.1. Study sites

This study was conducted in two Mediterranean shallow CO₂ vents located at: i) Milos Island (south-west Cyclades, Greece) and ii) Vulcano Island (south Italy) (Fig. 1). Populations of *Cymodocea nodosa* (Ucria) Asch. are found in both vents (Aliani et al., 1998; Apostolaki et al., 2014; De Biasi et al., 2004; Vizzini et al., 2013).

The Island of Milos is characterized by submerged volcanic activity. Sampling took place at Paleohori Bay (24°33.00' E; 36°40.00' N). Intense hydrothermal activity is evident around the whole bay (Thiermann et al., 1997), where mean gas composition was 92.5% CO₂, 0.13% O₂, 0.67% N₂, 7 ppm He, 11450 ppm H₂, 0.7 ppm CO and 916 ppm CH₄ in 2011 (Bayraktarov et al., 2013). A specific zonation of different deposits is evident in the sediment around the emission points. Adjacent to the vent, there is a yellow-orange deposit of arsenic sulfide minerals, where pore water pH reaches values down to 4.6 and temperature values up to 95 °C (Price et al., 2013a). This region is then followed by one of white precipitates of amorphous silica and native sulfur with microbial mats present. This region is followed by a ‘transition zone’ of grey sediment. Lastly, a ‘brown zone’ of unaffected brown sediment is observed, where temperature and pH progressively reach ambient values (Bayraktarov et al., 2013). While to our knowledge no literature data are available, a preliminary survey revealed an enrichment in a few trace elements (e.g., vanadium, mercury and above all arsenic) in sediment close to the volcanic emission points (Vizzini, unpublished data).

Similarly, the Island of Vulcano hosts a shallow hydrothermal

system of volcanic origin, which includes several sites of gas emission. In particular, at Levante Bay (14°57.60' E; 38°25.05' N), on the north-east side of the island, there is a main venting site where gas bubbles are made up almost entirely of CO₂ (97–99%, for details see Capaccioni et al., 2001; Boatta et al., 2013). Previous studies reported a pH gradient occurring along the bay with values ranging from 8.1 to 8.2 to 7.4–7.5 (Boatta et al., 2013; Horwitz et al., 2015). Due to the input of metal-rich volcanic fluids and the seawater features (low pH and Eh values) that affect the solubility and bioavailability of metals and metalloids, trace element concentration differs at a small spatial scale (Vizzini et al., 2013). Some elements (As, Ba, Hg, Mo, Ni, Pb, Zn) are more concentrated close to the primary vent, while others (Cd, Co, Cr, Cu, Fe, Mn, V) in sites at a distance of about 100–150 m upwards from the primary vent. Overall, the bay has low contamination levels with moderate potential for adverse biological effects, especially in the area between about 150 and 350 m from the primary vent, where localized detrimental effects on biota may occur (Vizzini et al., 2013).

In each site, plant and sediment properties and carbon content in seagrass meadows were investigated at two stations: one is far from the volcanic vents (~1.8 km at Milos, ~500 m at Vulcano) and is characterized by normal pCO₂ and pH (hereafter control) and the other is at the closest distance possible where seagrasses are found (~600 m at Milos, ~200 m at Vulcano) and is characterized by high pCO₂ and low pH (hereafter low pH) (Table 1). Both stations have similar water depths within each site (about 7–8 m depth at Milos and 1–2 m at Vulcano) and exposure (Fig. 1). Sampling was conducted in May 2013.

2.2. Field and laboratory analysis

2.2.1. Plant properties

Cymodocea nodosa shoots were collected with PVC hand-corers (Ø 10 cm, depth 20 cm) in three random replicates. The number of shoots was counted and above- (leaf blades and sheaths) and belowground (rhizomes, roots) tissues separated, dried at 60 °C and weighed. Leaf height and width were measured in a subsample of 20 shoots per replicate and the leaf area index (LAI) calculated as the product of mean leaf area per shoot times the shoot density. Carbon content (%) and δ¹³C (‰) were measured in dried seagrass tissues (3 mg), after treatment with HCl (2 N) to remove carbonates, using an elemental analyser (Thermo Flash EA 1112) interfaced to an isotope ratio mass spectrometer (Thermo Delta Plus XP). The analytical precision of the measurements based on the standard deviation of replicates of internal standards was 0.1‰.

Carbon stock in the living tissue component (leaf blades, leaf sheaths, rhizomes or roots) was first quantified in g C m⁻² using the following formula:

$$C_{stock, living\ tissues} = biomass\ (g\ m^{-2}) \times \%C / 100$$

and then converted into Mg ha⁻².

2.2.2. Sediment properties

Undisturbed surface sediment samples were collected by divers with PVC hand-corers (Ø 4 cm) in random triplicate within the seagrass meadow. Only the top 2 cm in the case of Vulcano and 1 cm in the case of Milos were sectioned for analysis.

Dry bulk density (DBD) was calculated as the dry weight of the sediment (60 °C for 48 h) divided by the volume of the original sediment sample. Porosity (ϕ) was obtained through the following formula:

$$\phi = \beta \times DBD / 100$$

where β is percentage water content of sediment estimated as:

$$\beta = [(wet\ weight - dry\ weight) / wet\ weight] \times 100$$

Before grain size analysis, bulk sediment was pretreated with hydrogen peroxide and Na-hexametaphosphate to eliminate organic

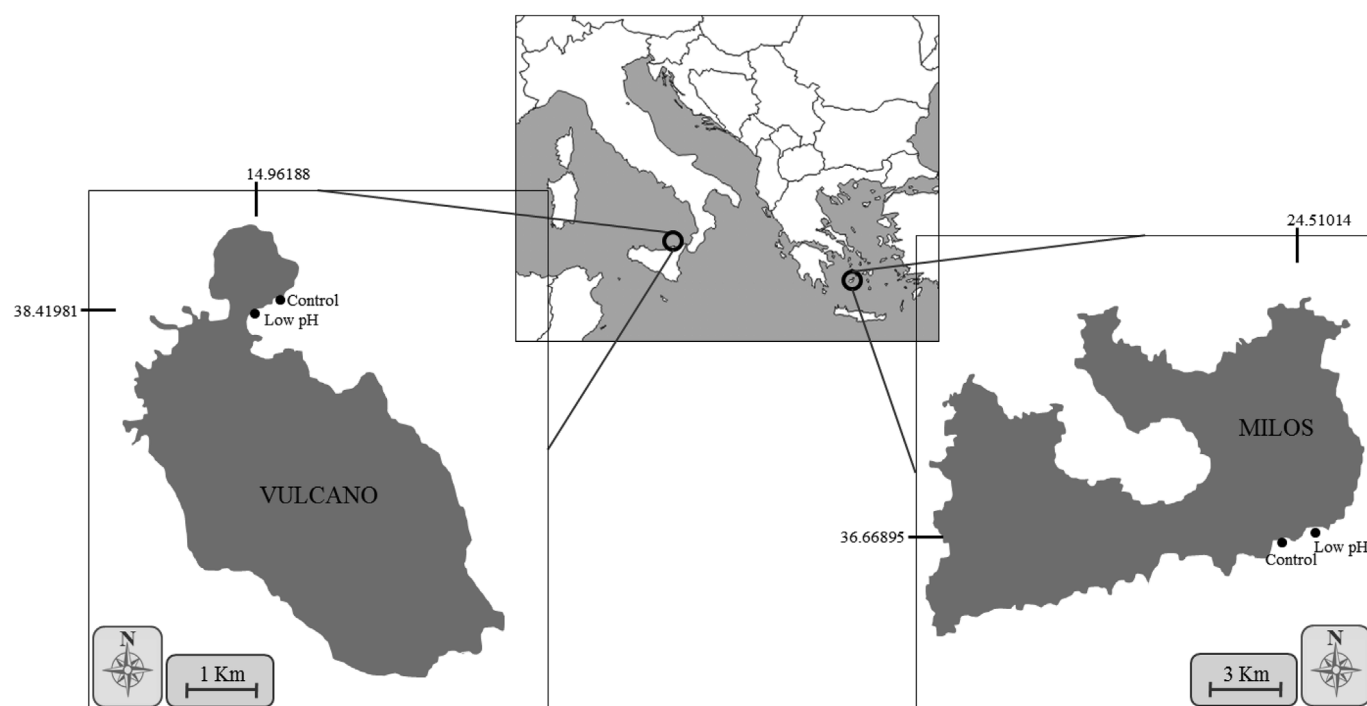


Fig. 1. Study sites (Milos and Vulcano Islands) showing the stations (control and low pH) where samples were collected (Windows Power Point, 2013 drawing based on maps from ArcGIS vers. 9.1).

Table 1

Seawater carbonate chemistry at the control and low pH of both Milos and Vulcano (ranges or means \pm SD). Data of Milos are taken from Misha et al. (unpublished), Bayraktarov et al., (2013), Price et al., (2013b), while those of Vulcano from Milazzo et al., (2016).

Site	Milos		Vulcano	
Station	Control	Low pH	Control	Low pH
pH	8.20 \pm 0.01	7.90 \pm 0.01	8.18 \pm 0.01	7.83 \pm 0.05
$p\text{CO}_2$ (μatm)	402.9 \pm 1.1	884.3 \pm 3.1	421 \pm 15	1180 \pm 153
TA (mmol kg^{-1})	2.7–3.6	2.1–2.2	2.5	2.5

matter and avoid particle flocculation. Sediment was then wet sieved through a 63 μm net, and the remaining fraction electro-mechanically sieved through a DIN ISO 3310-1 standard sieve set.

For chemical analysis (carbon content and $\delta^{13}\text{C}$), samples were oven dried (60 $^\circ\text{C}$ for 48 h) and ground in a ball mill (Retch MM200). Then samples were weighed (10 mg) in silver capsules and analysed using an elemental analyser (Thermo Flash EA 1112) for carbon content analysis: total carbon (TC) was analysed in sediment as it is, while, for organic carbon (OC) determination, sediment was acidified with HCl (2N) to remove carbonates. Inorganic carbon (IC) was estimated as the difference between TC and OC. $\delta^{13}\text{C}$ was analysed in acidified (HCl, 2N) sediment weighed (10 mg) in tin capsules using an isotope ratio mass spectrometer (Thermo Delta Plus XP).

Organic and inorganic carbon accumulations (respectively OC and IC stock) were calculated for the top sediment layer sampled by using the following equations:

$$\text{OC stock} = \text{DBD} (\text{g cm}^{-3}) \times \text{Slice} (\text{cm}) \times \text{OC} (\%) / 100$$

$$\text{IC stock} = \text{DBD} (\text{g cm}^{-3}) \times \text{Slice} (\text{cm}) \times \text{IC} (\%) / 100$$

and then converted into Mg ha^{-2} . At both sites, the stocks were calculated for the first 2 cm of sediment, assuming for the second cm of Milos 's stations the same carbon content and DBD as the first cm. OC and IC stocks were summed to obtain total carbon (TC stock).

2.2.3. Potential sources for sedimentary organic carbon

Organic matter sources potentially contributing to sedimentary organic carbon were collected in triplicate. In Milos, sources were represented by i) *C. nodosa*, ii) suspended particulate organic matter (POM), iii) volcanic-derived microbial mats; no macroalgae were present. At Vulcano, organic matter sources also included macroalgal species present at both control and low pH (namely: *Cystoseira compressa*, *Dyctiota dichotoma* and unidentified filamentous algae).

Microbial mats were collected close to the primary vent in Vulcano, as a volcanic vent end-member, by scraping the top layer of the mat-covered rocks with a ceramic knife; these data were also used for the Milos dataset. Macroalgae and the seagrass *C. nodosa* leaves were picked by hand, epiphytes removed by gentle scraping. Surface seawater for the isotopic analysis of the suspended particulate organic matter (POM) from the primary vent, and from the control was collected using 10 l bottles, and filtered through pre-combusted (450 $^\circ\text{C}$, 4 h) Whatman GF/F filters (0.45 μm). POM collection was not carried out in Milos Island for logistic reasons. Prior to $\delta^{13}\text{C}$ analysis, all samples were acidified (HCl, 2N) to remove carbonates, dried and weighed in tin capsules. Isotopic analysis was performed in an isotope ratio mass spectrometer (Thermo Delta Plus XP).

2.3. Data analysis

Analysis of differences in seagrass and sediment properties were performed through two-way ANOVA and factors tested were: station (2 levels: control and low pH) and site (2 levels: Milos and Vulcano). ANOVA was not run on leaf width data as no variability among replicates was detected and on silt and clay (%) data as only one replicate was collected in Milos. Linear regression was used to detect relationships between seagrass and sediment features and TC_{stock} within each site. Prior to ANOVA and linear regression analyses, all data were tested for normality and homoscedasticity using, respectively, Shapiro-Wilk and Cochran's tests. When significant differences occurred, appropriate means compared using post-hoc Student–Newman–Keuls test.

Bayesian mixing models (package SIAR: Stable Isotope Analysis in R; Parnell et al., 2010) were used to investigate the origin of OC present

in sediments by quantifying the relative proportion of potential sources to the isotopic composition of the sediment. $\delta^{13}\text{C}$ was used as a variable and the number of sources considered was chosen according to the stations and sites and included potential end-members that could contribute to the carbon isotopic signature of the sediment (i.e., macrophytes, suspended particulate organic matter [POM] and volcanic-derived microbial mats, see the section above for details). As POM was collected only in Vulcano Island, data used for Milos are those available from other vents [Panarea (Vizzini unpublished data) and Vulcano (this study) Islands, Aeolian Archipelago, Italy]]. Mixing models were run separately for each station and site. Macroalgae of which the biomass was very abundant in Vulcano (*C. compressa* and *D. dichotoma*) were combined together in a group prior to the model, due to the absence of significant differences among their $\delta^{13}\text{C}$ signature (Phillips et al., 2005) (ANOVA, $df = 1$, Pseudo- $F = 0.0052$, $p = 0.940$) to reduce sources of uncertainty that may influence mixing model results when using multiple end-members (Phillips et al., 2014).

3. Results

3.1. Seagrass properties

Differences in *Cymodocea nodosa* features between stations (control and low pH) were not consistent in the two sites (Milos and Vulcano), as for most variables the interaction term “station x site” had a significant effect (Table 2). This was the case for leaf length, leaf area per shoot, leaf area index (LAI) and aboveground biomass, which were significantly lower at the control than at the low pH in Milos, whereas the opposite trend occurred in Vulcano with significant differences only in two cases (leaf area and LAI). Density and belowground biomass differed only for the factor site with values lower in Milos than in Vulcano.

TC stock in living tissues was significantly lower at both stations in Milos than in Vulcano, and significantly higher at the low pH than at the control in Milos (Table 2).

3.2. Sediment properties

Sediment features did not vary between stations at both sites (Table 3). On the contrary, differences between sites were recorded for DBD, porosity and $\delta^{13}\text{C}$, with Milos showing higher values than Vulcano for porosity and $\delta^{13}\text{C}$, while a significant effect of the interaction term “station x site” was obtained for only C/N ratios with overall higher values at the control than at low pH.

OC stock and IC stock significantly decreased from control to low pH (2-way ANOVA: factor station $F_{1,8} = 5.88$, $p = 0.042$ and $F_{1,8} = 17.64$, $p = 0.003$ respectively) (Fig. 2). Accordingly, TC stock significantly decreased at low pH ($F_{1,8} = 15.70$, $p = 0.004$) and the same also occurred summing up the C stock in the living tissues and in the sediment ($F_{1,8} = 7.07$, $p = 0.029$).

Linear regression analysis showed a significant positive correlation between porosity and TC in Vulcano (*adjusted R-square* = 0.728, $p = 0.019$) and a negative correlation between aboveground biomass and TC stock only in Milos (*adjusted R-squared* = 0.887, $p = 0.018$). Both belowground biomass and seagrass density were not correlated with TC stock in both sites ($p > 0.05$).

Organic matter sources used in the mixing models are showed in Table 4. According to the mixing model estimation, most sources showed wide and overlapping contributions (Fig. 3). Organic carbon of sediment seemed to be mainly derived from POM above all at Milos (range low95%-high95% = 0.65–0.89 and 0.04–0.60 at control and low pH respectively). *C. nodosa* showed similar ranges in both stations of the two sites (range low95% = 0.00–0.31; range high95% = 0.34–0.42). The same was also found for the contribution of volcanic-derived microbial mats (range low95%-high95% = 0.05–0.56 and 0.01–0.54 at Milos and Vulcano respectively).

Table 2
Data of *C. nodosa* properties (mean \pm SD) at the stations (control and low pH) and sites (Milos and Vulcano) investigated and relative statistical results (2-way ANOVA, factors: station and site, and post-hoc tests). Living tissues include leaf blades, leaf sheaths, rhizomes and roots. Leaf width was not statistically analysed because it was identical in each pH condition (control and low pH) and site. M: Milos, V: Vulcano, C: control, L: low pH.

Station	Milos		Vulcano		2-way ANOVA		post-hoc Student–Newman–Keuls test	
	Control	Low pH	Control	Low pH				
Density (shoots m^{-2})	122.67 \pm 72.1	208.0 \pm 112.0	525.0 \pm 139.2	616.67 \pm 202.1	site: $F_{1,8} = 25.31$, $p = 0.001$		M < V	
Leaf length (cm)	10.07 \pm 1.40	18.70 \pm 2.90	10.12 \pm 1.39	7.10 \pm 0.38	station x site: $F_{1,8} = 32.76$, $p = 0.0004$		MC < ML, VL = VC, VL < ML, MC = VC	
Leaf width (cm)	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0				
Leaf area (cm^2 shoot $^{-1}$)	7.68 \pm 1.31	13.65 \pm 2.06	7.96 \pm 0.59	4.94 \pm 0.08	station x site: $F_{1,8} = 38.46$, $p = 0.003$		MC < ML, VL < VC, VL < ML, MC = VC	
LAI (m^2 leaves m^{-2})	0.094 \pm 0.016	0.284 \pm 0.043	0.418 \pm 0.031	0.305 \pm 0.005	station x site: $F_{1,8} = 94.10$, $p = 0.0001$		MC < ML, VL < VC, ML = VL, MC < VC	
Aboveground biomass (g DW m^{-2})	3.89 \pm 0.56	10.94 \pm 2.00	30.33 \pm 3.20	27.52 \pm 1.95	station x site: $F_{1,8} = 94.10$, $p = 0.0004$		MC < ML, VL = VC, ML < VL, MC < VC	
Belowground biomass (g DW m^{-2})	12.22 \pm 2.86	16.28 \pm 2.25	23.06 \pm 3.90	27.23 \pm 8.55	site: $F_{1,8} = 14.02$, $p = 0.006$		M < V	
TC stock living tissues (Mg C ha^{-2})	0.05 \pm 0.01	0.09 \pm 0.01	0.21 \pm 0.01	0.21 \pm 0.04	#station x site: $F_{1,8} = 13.86$, $p = 0.006$		MC < ML, VC = VL, ML < VL, MC < VC	

data Ln transformed.

Table 3

Data of sediment properties (mean \pm SD) at the stations (control and low pH) and sites (Milos and Vulcano) investigated and relative statistical results (2-way ANOVA, factors: station and site, and post-hoc tests). Statistical significance in silt and clay content could not be tested, due to lack of replicates in Milos. M: Milos, V: Vulcano, C: control, L: low pH.

Site	Milos		Vulcano		2-way ANOVA	post-hoc Student–Newman–Keuls test
Station	Control	Low pH	Control	Low pH		
DBD (g cm^{-3})	1.20 \pm 0.05	1.25 \pm 0.04	1.55 \pm 0.02	1.54 \pm 0.06	site: $F_{1,8} = 155.24$, $p = 0.0001$	M < V
Porosity (ϕ)	0.42 \pm 0.01	0.44 \pm 0.02	0.42 \pm 0.03	0.38 \pm 0.01	site: $F_{1,8} = 7.81$, $p = 0.023$	V < M
Silt and clay (%)	2.5	2.8	1.0 \pm 0.1	1.4 \pm 0.5	–	–
$\delta^{13}\text{C}$ (‰)	–18.87 \pm 0.15	–18.75 \pm 0.04	–21.94 \pm 0.21	–22.69 \pm 1.94	site: $F_{1,8} = 38.32$, $p = 0.003$	V < M
C/N	6.90 \pm 0.99	5.73 \pm 0.76	10.54 \pm 1.72	3.66 \pm 2.48	station x site: $F_{1,8} = 9.14$, $p = 0.016$	ML = MC, VL < VC, VL = ML, MC < VC

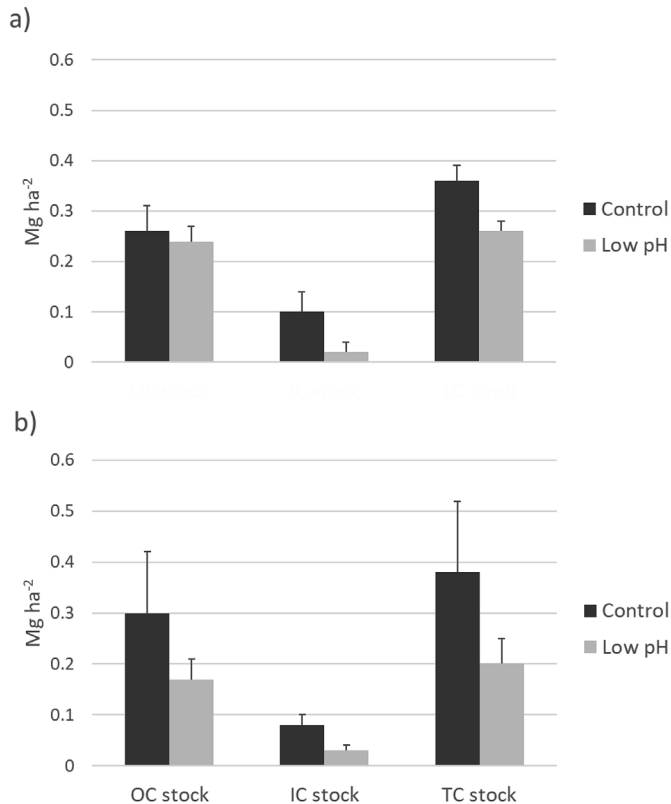


Fig. 2. Organic, inorganic and total carbon stocks (OC, IC and TC, Mg ha^{-2} , mean \pm SD) in surficial sediment (2 cm) at the stations (control and low pH) and sites (a) Milos and (b) Vulcano) investigated.

4. Discussion

This study focused on the seagrass *Cymodocea nodosa*, one of the four native seagrass species found in the Mediterranean Sea (Luisetti et al., 2013) and investigated both seagrass and sediment features in

different $p\text{CO}_2$ -low pH conditions occurring in two Mediterranean volcanic vents with potential implications for carbon sequestration and storage capacity in future acidified ocean.

Looking at seagrass properties, no consistency was found between the two different and geographically distinct venting sites, since highly variable results between sites did not allow generalization of the observed trend. The increase in a number of seagrass features observed in Milos at low pH is consistent with previous studies in other venting sites (Hall-Spencer et al., 2008; Russell et al., 2013; Takahashi et al., 2016), but is inconsistent with those from Vulcano Island, where a different trend was in fact observed, in agreement with previous findings in the area (Apostolaki et al., 2014; Vizzini et al., 2017). In more detail, at low pH in Milos we observed a three-fold increase in the aboveground biomass and a two-fold increase in ratio of above to belowground biomass, while at Vulcano no significant variation occurred between control and low pH station.

Consistent with aboveground biomass, leaf area and LAI showed the reverse pattern between the vent sites, increasing at low pH in Milos and decreasing at low pH in Vulcano compared to the corresponding controls. Experimental evidence has shown a positive correlation between LAI and pH in natural conditions, suggesting that dense meadows with big leaves have a high capacity to buffer pH (Hendriks et al., 2014). Our results, however, imply that the relationship between changes in seawater chemistry and structural variables of the meadow in naturally acidified conditions may not be straightforward.

It has been suggested before that the response of seagrasses to naturally increased CO_2 levels is species-specific, with species showing an increase, decrease or no variation in terms of biomass (Russell et al., 2013; Takahashi et al., 2016). Here, we provide evidence that seagrass response is also site-specific, as the same species (*C. nodosa*) seems to have a different structure at the meadow level (i.e., biomass, LAI) in the low pH stations in comparison with controls in the two venting sites. More effort, however, is needed to widen spatial replication within each site, in order to gain a more reliable picture.

The two sites were more consistent when looking at sediment properties, showing decreased carbon accumulation in surficial sediment at low pH stations. As only surficial sediment was collected, and carbon content and sediment density can change with depth (e.g.,

Table 4

$\delta^{13}\text{C}$ data (‰; mean \pm SD) of sources potentially contributing to sedimentary carbon used in the mixing models carried out separately for each station (control and low pH) and site (Milos and Vulcano). POM (suspended particulate organic matter) data for Milos are averaged values collected from other volcanic vents [Panarea (Vizzini unpublished data) and Vulcano (this study) Islands, Aeolian Archipelago, Italy].

Site Station	Milos		Vulcano	
	Control	Low pH	Control	Low pH
<i>Cymodocea nodosa</i>	–8.82 \pm 0.17	–7.56 \pm 0.04	–9.19 \pm 0.17	–13.12 \pm 0.05
POM	–21.71 \pm 0.89	–24.39 \pm 0.79	–20.87 \pm 1.05	–24.76 \pm 0.14
Macroalgae	–	–	–	–19.75 \pm 0.77
Filamentous algae	–	–	–23.44 \pm 0.17	–
Microbial mats	–	–26.11 \pm 0.91	–	–26.11 \pm 0.91

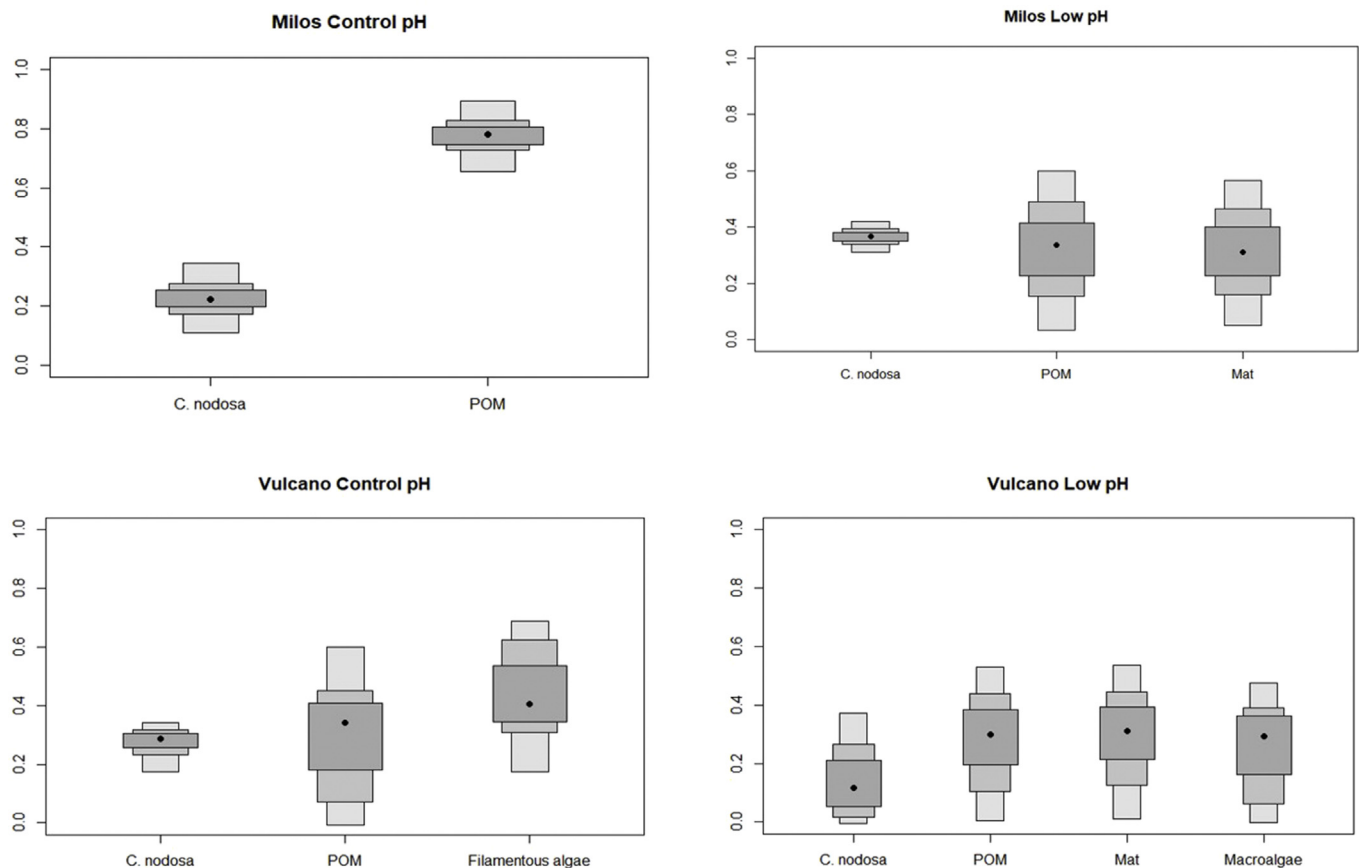


Fig. 3. Mixing models output showing the contribution of different organic matter sources to sediment organic carbon at the stations (control and low pH) and sites (Milos and Vulcano) investigated. Boxplots illustrate the 95%, 75% and 50% confidence intervals from light to dark and dots indicate the mode. POM: suspended particulate organic matter; Mat: microbial mats.

Lavery et al., 2013; Marbà et al., 2015), the patterns discussed cannot straightforwardly be expanded to deeper layers of sediment and more investigation is needed to have a more comprehensive picture. So far, not much attention has been given to the capacity of *C. nodosa* to accumulate carbon both in living biomass and sediments, as no study has measured the carbon stocks in *C. nodosa* meadows (but Apostolaki et al. under revision). Only a few studies have indirectly assessed carbon sequestration by measuring the carbon metabolism of the meadows (Barrón et al., 2004; Cebrián et al., 1997; Duarte et al., 2005). As carbon storage in vegetated habitats depends, among other factors, also on the seagrass species involved (Lavery et al., 2013), the lack of other studies on *C. nodosa* prevented any comparison with the literature.

Here, the decrease in IC surficial stock may be related to the higher solubility of carbonates. This was an expected trend, typical of vent systems, and strictly correlated to the fact that the solubility of calcium carbonate depends strongly on pH. The intense seagrass metabolism (Apostolaki et al., 2014), photosynthesis but also respiration with consequent CO₂ release, may additionally impede the accumulation of carbonates in seagrass sediments in these particular environments, reducing the IC stock. IC stock was low also in the controls, probably depending on the mineral composition of sediments in volcanic islands.

In terms of OC, we may reasonably assume that seagrass properties at Vulcano Island (such as leaf area, LAI and biomass), which appeared negatively affected (although not significantly for biomass) by environmental conditions at the low pH station, may prevent an increase in carbon accumulation in sediments. The reduced leaf area and biomass close to the vents at Vulcano may be directly related to both the higher grazing pressure of consumers on *C. nodosa* at low pH, as previously hypothesized (Apostolaki et al., 2014), and/or the influence of contaminants in the area. Indeed, sediment pollution indices revealed

that detrimental effects on the biota may occur in the area between about 150 and 350 m from the primary vent (Vizzini et al., 2013). Altogether, these peculiarities in the environment, sediment and seagrass features in the vents, cannot be directly related to high pCO₂, but also to the biogeochemical processes involved, such as low pH and redox potential (Eh), which enhance trace element precipitation at the sediment-water interface (Vizzini et al., 2013).

While at Vulcano the changes in structural features of the meadow would entail the overall reduction in carbon content and surficial accumulation, at Milos a generally less straightforward pattern is suggestive of a more complex story. In fact, the increase in seagrass aboveground biomass, leaf length, leaf area and LAI close to the vents would imply a greater potential to trap particles and to accumulate organic carbon in sediments, which however did not occur. This could be related to different factors and, firstly to the seagrass species itself. Previous studies, have highlighted a strong variability in the capacity to stock carbon among different seagrass species. Lavery et al. (2013), for example, suggested that larger seagrasses (i.e., *Posidonia australis*) can stock more carbon, efficiently trapping particles inside their beds. Accordingly, the weak capacity of *C. nodosa* to retain sediments may be related to the shortness of the leaves and low belowground biomass in comparison with other seagrass species (Russell et al., 2013), as well as to their subsequent weak entrapping and compacting role, respectively. At low density (as in the case of both control and low pH stations at Milos), *C. nodosa* is expected not to trap carbon, both autochthonous and allochthonous, in an efficient way and the carbon trapped may be easily subject to resuspension and reduction because of the impact of local hydrodynamism on low-density meadows. The belowground compartments of *C. nodosa* are generally small and the meadows not perennial; consequently, the possibility of burying large quantities of

carbon could be greatly affected. The particular seagrass features may reduce the capacity to trap carbon cancelling out the potential benefit deriving from increased CO₂. Recently, Mazarrasa et al. (2018) reviewed both the biotic and abiotic factors that affect long-term OC sequestration in seagrass habitats, suggesting that short-leaf, low density and low canopy complexity of the meadows do not drive large accumulation of carbon in sediments, and that biotic factors combine with abiotic ones (i.e., turbidity, nutrient availability, depth and hydrodynamics) in determining the carbon sequestration capacity of seagrasses. In the present study, control and low pH stations were selected in order to be comparable in terms of the main abiotic factors (i.e., depth, exposure and nutrient availability) within each vent to avoid any possible effect when contrasting the effect of vent conditions on carbon accumulation in sediment. Moreover, sediment features can also influence carbon accumulation potential in sediment and further prevent a high carbon burial at low pH stations. A recent study by Gullström et al. (2018) found out that C stocks in seagrass habitats are predicted primarily by sediment density and only to a lesser extent by seagrass features. In the present study, while density did not show any pattern, sediment porosity was correlated with TC stock in surficial sediment from Vulcano. In particular, a lower capacity to accumulate carbon corresponded to lower porosity (at low pH station), supporting the findings that abiotic factors may influence the carbon storage capacity of the seagrass (Dahl et al., 2016).

The Bayesian mixing model outcome provided the ranges of possible contributions of sources to sedimentary carbon, and although they were wide and often overlapped, indicating a degree of uncertainty in the models, which however is intrinsic in ecological systems (Parnell et al., 2010), the contribution of *C. nodosa* appeared not much high, also at Milos where *C. nodosa* biomass and density were lower and POM assumed a dominant role in contributing to the organic matter of the sediment. In addition, the particular origin of sedimentary C at the low pH station is corroborated by the contribution of volcanic-derived microbial mats, responsible for the labilisation of sedimentary organic matter resulting from the decreased C/N ratios, above all at Vulcano.

Although carbon sinks may depend strongly on the regional location of the study area (Gullström et al., 2018) and geographical variability occurs in seagrass capacity to stock carbon in sediments (Miyajima et al., 2015), the consistency of results from the two vents suggests that, probably through different underlying mechanisms, C content and accumulation potential in *C. nodosa* sediments may not increase at high pCO₂-low pH conditions and that the prediction of positive effects of OA on seagrasses and on their capacity to stock carbon (Garrard and Beaumont, 2014) requires further investigation.

5. Conclusions

Recent studies have provided evidence of increased productivity, biomass and density of seagrasses under high pCO₂-low pH conditions (Russell et al., 2013; Takahashi et al., 2016), and suggested a positive relationship between ocean acidification and carbon stock capacity of marine vegetation (Garrard and Beaumont, 2014; Mazarrasa et al., 2018). The relatively simplified field observations of this study provided evidence of a non-consistent response of the seagrass *Cymodocea nodosa* in the two vents and an overall decrease in carbon surficial stocks at high pCO₂-low pH conditions. Although the assessment has been carried out only in surficial sediment, the results from two different volcanic vents contribute to the development of conceptual models of the changes expected in future high pCO₂-low pH oceans. There is also evidence that the intrinsic features of each marine site and seagrass bed should be carefully taken into account when studying carbon accumulation, and generalizing the potential effects that acidified oceans may have on the corresponding carbon stocking capacity is challenging. Moreover, while CO₂ vents are used as a proxy of real long-term and chronic exposure to high pCO₂-low pH within realistic natural conditions (Fabricius et al., 2011; Takahashi et al., 2016), caution

should be taken because of the limitations that are typical of field studies represented mainly by the unpredicted variability of biotic and abiotic factors, specifically by the high variability of pCO₂, and/or the bias of other variables (e.g., H₂S, metals).

Scientific interest in the potential of marine vegetation as mitigation service to absorb and stock excessive carbon is growing (Russell et al., 2013). Considering this ecosystem service in relation to global climate change and, in particular, to the acidification of the seawater is highly important. Further studies are required to collect similar data from different natural CO₂ vent systems where seagrasses thrive and to analyze deeper sediment layers and larger sample sizes in order to provide a more comprehensive analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2019.03.001>.

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